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(21) International Application Number: PCT/US99/29958 (22) International Filing Date: 16 December 1999 (16.12.99) (30) Priority Data: 60/112,448                      16 December 1998 (16.12.98)      US (71) Applicants (for all designated States except US): NORTH-WESTERN UNIVERSITY [US/US]; 1801 Maple Avenue, Evanston, IL 60201-3135 (US). UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA [IT/IT]; Via Campi, 183, I-41100 Modena (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): SHOICHET, Brian, K. [US/US]; 2650 N. Lakeview, #2309, Chicago, IL 60614 (US). COSTI, Maria, Paola [IT/IT]; Via Campi 183, I-41100 Modena (IT). TONDI, Donatella [IT/IT]; Via Campi 183, I-41100 Modena (IT). (74) Agents: CROOK, Wannell, M. et al.; Sheridan Ross P.C., Suite 1200, 1560 Broadway, Denver, CO 80202-5141 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report.	
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(57) Abstract			
<p>The invention provides novel non-<math>\beta</math>-lactam inhibitors of <math>\beta</math>-lactamases. In particular, the invention provides such inhibitors which are boronic acids of formula (1) which is set forth in the specification. These compounds may be used with <math>\beta</math>-lactam antibiotics to treat <math>\beta</math>-lactam-antibiotic-resistant bacterial infections. Finally, the invention provides a pharmaceutical composition comprising these compounds.</p>			
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INHIBITORS OF  $\beta$ -LACTAMASES AND USES THEREFORBACKGROUND

Bacterial resistance to antibiotics has raised fears of an approaching medical catastrophe (Neu, *Science*, 257, 1064-1073 (1992)). Evolutionary selection and genetic transformation have made this problem pressing. Most antibiotic drugs are derivatives of naturally occurring bactericides (Davies, *Science*, 264, 375-382 (1994)), and many resistance mechanisms evolved long ago. Human use of antibiotics has refined these mechanisms and promoted their spread through gene transfer (Davies, *Science*, 264, 375-382 (1994)). A resistance mechanism originating in one species of bacteria can be expected to spread throughout the biosphere.

Bacterial adaptations to  $\beta$ -lactam drugs (e.g., amoxicillin, cephalothin, clavulanate, aztreonam) are among the best studied and most pernicious forms of antibiotic resistance.  $\beta$ -lactams target enzymes that are unique to bacteria and are thus highly selective. They have been widely prescribed. In the absence of resistance,  $\beta$ -lactams are the first choice for treatment in 45 of 78 common bacterial infections (*Goodman & Gilman's The Pharmacological Basis of Therapeutics* (Hardman et al., eds., McGraw-Hill, New York, 1996)). The evolution of resistance to these drugs has raised the cost of antibiotic therapy and reduced its effectiveness, leading to increased rates of morbidity and mortality.

$\beta$ -lactam antibiotics inhibit bacterial cell wall biosynthesis (Tomasz, *Rev. Infect. Dis.*, 8, S270-S278 (1986)). The drugs form covalent complexes with a group of transpeptidases/carboxypeptidases called penicillin binding proteins (PBPs). PBP inactivation disrupts cell wall biosynthesis, leading to self-lysis and death of the bacteria.

Bacteria use several different mechanisms to escape from  $\beta$ -lactam drugs (Sanders, *Clinical Infectious Disease*, 14, 1089-1099 (1992); Li et al., *Antimicrob. Agents Chemother.*, 39, 1948-1953 (1995)). Probably the most widespread is the hydrolysis of  $\beta$ -lactams by  $\beta$ -lactamase enzymes.

TEM-1 and AmpC are two  $\beta$ -lactamases from *Escherichia coli*. *E. coli* is an important pathogen in its own right. It is the most common cause of gram-negative bacterial infection in humans (Levine, *New Engl. J. Med.*, 313, 445-447 (1985)), and is the most prevalent hospital-acquired infection (Thornsberry, *Pharmacotherapy*, 15, S3-8 (1995)). *E. coli* that carry TEM-1, or for which AmpC production has been derepressed, are resistant to  $\beta$ -lactam treatment. As of 1992, as many as 30% of community-isolated

*E. coli* and 40-50% of hospital-acquired *E. coli* in the United States were resistant to  $\beta$ -lactams such as amoxicillin (Neu, *Science*, 257, 1064-1073 (1992)). Many of these resistant *E. coli* are resistant to  $\beta$ -lactamase inhibitors such as clavulanic acid and sulbactam.

5           TEM-1 and AmpC are major forms of plasmid-based and chromosomal  $\beta$ -lactamases and are responsible for resistance in a broad host range. The versions of TEM and AmpC (Galleni, et al., *Biochem. J.*, 250, 753-760 (1988)) in other bacterial species share high sequence identity to TEM-1 and AmpC from *E. coli*. TEM-1 structurally and catalytically resembles the class A  $\beta$ -lactamase from *Staphylococcus aureus*.  
10           The structures of AmpC from *Citrobacter freundii* and *Enterobacter cloacae* have been determined, and they closely resemble the structure of the *E. coli* enzyme (Usher et al., *Biochemistry*, 37, 16082-16092 (1998)).

          To overcome the action of  $\beta$ -lactamases, medicinal chemists have introduced compounds that inhibit these enzymes, such as clavulanic acid, or compounds that are less  
15           susceptible to enzyme hydrolysis, such as aztreonam. Both have been widely used in antibiotic therapy (Rolinson, *Rev. Infect. Diseases* 13, S727-732 (1991)); both are  $\beta$ -lactams. Their similarity to the drugs that they are meant to protect or replace has allowed bacteria to evolve further, maintaining their resistance.

          Resistance to these new classes of  $\beta$ -lactams has arisen through modifications of  
20           previously successful mechanisms. Point substitutions in  $\beta$ -lactamases allow the enzymes to hydrolyze compounds designed to evade them (Philippon et al., *Antimicrob. Agents Chemother.*, 33, 1131-1136 (1989)). Other substitutions reduce the affinity of  $\beta$ -lactam inhibitors for the enzymes (Saves, et al., *J. Biol. Chem.*, 270, 18240-18245 (1995)) or allow the enzymes to simply hydrolyze them. Several gram positive bacteria, such as  
25           *Staph. aureus*, have acquired sensor proteins that detect  $\beta$ -lactams in the environment of the cell (Bennet and Chopra, *Antimicrob. Agents Chemotherapy*, 37, 153-158 (1993)).  $\beta$ -lactam binding to these sensors leads to transcriptional up-regulation of the  $\beta$ -lactamase.  $\beta$ -lactam inhibitors of  $\beta$ -lactamases, thus, can induce the production of the enzyme that they are meant to inhibit, defeating themselves.

30           It is noteworthy that the human therapeutic attack on bacteria has paralleled the path taken in nature. Several species of soil bacteria and fungi produce  $\beta$ -lactams,

presumably as weapons against other bacteria (although this remains a matter of debate). Over evolutionary time, susceptible bacteria have responded to  $\beta$ -lactams with  $\beta$ -lactamases, among other defenses. In turn, soil bacteria have produced  $\beta$ -lactams that resist hydrolysis by  $\beta$ -lactamases or have produced  $\beta$ -lactams that inhibit the  $\beta$ -lactamases.

5 *Streptomyces clavuligeris* makes several  $\beta$ -lactams, including clavulanic acid, a clinically used inhibitor of class A  $\beta$ -lactamases such as TEM-1. *Chromobacterium violaceum* makes aztreonam, a clinically used monobactam that resists hydrolysis by many  $\beta$ -lactamases. One reason why bacteria have been able to respond rapidly with "new" resistance mechanisms to  $\beta$ -lactams, and indeed many classes of antibiotics, is that the

10 mechanisms are not in fact new. As long as medicinal chemistry focuses on new  $\beta$ -lactam molecules to overcome  $\beta$ -lactamases, resistance can be expected to follow shortly. The logic will hold for any family of antibiotic where the lead drug, and resistance mechanisms to it, originated in the biosphere long before their human therapeutic use. This includes the aminoglycosides, chloramphenicol, the tetracyclines and vancomycin.

15 One way to avoid recapitulating this ancient "arms race" would be to develop inhibitors that have novel chemistries, dissimilar to  $\beta$ -lactams. These non- $\beta$ -lactam inhibitors would not themselves be degraded by  $\beta$ -lactamases, and mutations in the enzymes should not render them labile to hydrolysis. Novel inhibitors would escape detection by  $\beta$ -lactam sensor proteins that up-regulate  $\beta$ -lactamase transcription, and may

20 be unaffected by porin mutations that limit the access of  $\beta$ -lactams to PBPs. Such inhibitors would allow current  $\beta$ -lactam drugs to work against bacteria where  $\beta$ -lactamases provide the dominant resistance mechanism.

It has previously been reported that boric acid and certain phenyl boronic acids are inhibitors of certain  $\beta$ -lactamases. See, Kiener and Waley, *Biochem. J.*, **169**, 197-204

25 (1978) (boric acid, phenylboronic acid (2FDB) and *m*-aminophenylboronate (MAPB)); Beesley et al., *Biochem. J.*, **209**, 229-233 (1983) (twelve substituted phenylboronic acids, including 2-formylphenylboronate (2FORMB), 4-formylphenylboronate (4FORMB), and 4-methylphenylboronate (4MEPB)); Amicosante et al., *J. Chemotherapy*, **1**, 394-398 (1989) (boric acid, 2FDB, MAPB and tetraphenylboronic acid). More recently, *m*-

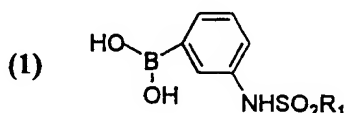
30 (dansylamidophenyl)-boronic acid (NSULFB) has been reported to be a submicromolar inhibitor of the *Enterobacter cloacae* P99  $\beta$ -lactamase. Dryjanski and Pratt,

*Biochemistry*, **34**, 3561-3568 (1995). In addition, Strynadka and colleagues used the crystallographic structure of a mutant TEM-1 enzyme-penicillin G complex to design a novel alkylboronic acid inhibitor [(1R)-1-acetamido-2-(3-carboxyphenyl)ethane boronic acid] with high affinity ( $K_i = 110$  nM) for this enzyme. Strynadka et al., *Nat. Struc. Biol.*, **3**, 688-695 (1996).

Finally, Weston et al. describe the testing of 37 boronic acids for inhibition of *E. coli* AmpC  $\beta$ -lactamase. Weston et al., *J. Med. Chem.*, **41**, 4577-4586 (1998). The activity of the compounds varied considerably, with benzo-[*b*]-thiophene-2-boronic acid (BZBTH2B) being the most potent inhibitor ( $K_i = 27$  nM). Using the previously-determined structure of the AmpC-MAPB complex (see Usher et al., *Biochem.*, **37**, 16082-16092 (1998)), several of the inhibitors were modeled into the AmpC binding site, and certain aspects of the interactions of the inhibitors with the enzyme were identified. The article concluded, however, that the modeling carried with it some ambiguities and that key questions regarding the structural bases for activity remained unanswered.

#### SUMMARY OF THE INVENTION

The invention provides non- $\beta$ -lactam inhibitors of  $\beta$ -lactamases. In particular, the invention provides  $\beta$ -lactamase inhibitors having the formula:



wherein:

$R_1$  is N-lower alkyl, a cyclic alkene or a heterocyclic alkene, wherein the cyclic alkene and heterocyclic alkene may be substituted with one or more substituents  $R_2$ ; and

each  $R_2$  is independently H, a halogen atom, lower, alkyl, lower alkyl substituted with one or more halogen atoms,  $NH_2$ , NO,  $NO_2$ , N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, COO-lower alkyl,  $CONH_2$ , CON-lower alkyl,

$\text{SO}_3\text{H}$ ,  $\text{SO}_2\text{NH}_2$ ,  $\text{SO}_2\text{N-lower alkyl}$ , or  $\text{B(OH)}_2$ , except that  $\text{R}_2$  cannot be N-lower alkyl when  $\text{R}_1$  is naphthalene.

The invention also provides a method of treating a  $\beta$ -lactam-antibiotic-resistant bacterial infection. The method comprises administering to an animal suffering from such an infection an effective amount of a  $\beta$ -lactamase inhibitor of formula (1), or a pharmaceutically-acceptable salt thereof, and an effective amount of a  $\beta$ -lactam antibiotic.

Finally, the invention provides pharmaceutical compositions comprising compounds of formula (1), or pharmaceutically-acceptable salts thereof, and a pharmaceutically-acceptable carrier. The pharmaceutical compositions may also comprise  $\beta$ -lactam antibiotics.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B. Diagrams of the synthesis of compounds of formula (1).

Figure 2: Diagram of scheme for the synthesis of compounds 15-26.

#### DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, the following terms have the following meanings.

"Lower alkyl" means a straight-chain or branched-chain alkyl containing 1-4 carbon atoms.

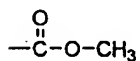
"N-lower alkyl" means N with one or more lower alkyls attached, such as  $-\text{NHCH}_3$  and  $-\text{N}(\text{CH}_3)_2$ .

"O-lower alkyl" means O with a lower alkyl attached, such as  $-\text{OCH}_3$ .

"CO-lower alkyl" means  $\text{C=O}$  with a lower alkyl attached to the C, such as  $-\text{COCH}_3$ .

"Lower alkyl-COOH" means  $-\text{COOH}$  preceded by a lower alkyl radical, such as  $-\text{CH}_2\text{COOH}$ .

"COO-lower alkyl" means  $-\text{COO}-$  having a lower alkyl attached to the O, such as



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"CON-lower alkyl" means CON having one or more lower alkyls attached to the N, such as  $\text{—}\overset{\text{O}}{\underset{\text{||}}{\text{C}}}\text{—NHCH}_3$ .

"SO<sub>2</sub>N-lower alkyl" means SO<sub>2</sub>N having one or more lower alkyls attached to the N, such as -SO<sub>2</sub>NHCH<sub>3</sub>.

5 "Cyclic alkene" means a structure containing 1 or 2 rings, each ring containing 5 or 6 carbon atoms and at least one double bond. One or both of the rings may be aromatic. If the cyclic alkene contains more than one ring, the rings may be fused, connected by a bond, or connected by a linker, L. Preferably, L is a short chain containing up to six atoms in the chain. Suitable linkers include -O-, -NH-, -S-, -SO<sub>2</sub>-, -N=N-, -SO<sub>2</sub>-  
10 NH-, -NH-CO-, -COO-, -CO-, lower alkyl radical (e.g., -CH<sub>2</sub>-), lower alkene (e.g., -CH=CH-), lower alkyne (e.g., -C≡C-), and combinations thereof.

"Heterocyclic alkene" means a cyclic alkene, as defined above, wherein one or both of the rings contain(s) one or more S, N or O atoms.

15 "Lower alkene" means a straight-chain or branched-chain alkene containing 2-4 carbon atoms.

"Lower alkyne" means a straight-chain or branched-chain alkyne containing 2-4 carbon atoms.

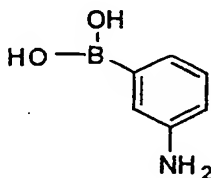
Compounds of formula (1) according to the invention include all optical isomers.

Preferred compounds of formula (1) are those wherein R<sub>1</sub> is an cyclic alkene or  
20 heterocyclic alkene containing 2 rings linked by a linker L. The preferred linkers are -SO<sub>2</sub>- and -NH-SO<sub>2</sub>-. Most preferred are compounds 1, 15 and 16 listed in Table 1B below. Also highly preferred are compounds 2 and 3 listed in Table 1B below.

The compounds of formula (1) can be synthesized as described below. Unless  
25 otherwise noted, the various chemicals used in the syntheses described below are available from commercial sources including Aldrich Chemical, Milwaukee, WI, Lancaster Synthesis, Windham, NH, TCI America, Portland, OR, Sigma Chemical Co., St. Louis, MO, Acros Organics, Pittsburgh, PA, Chemservice Inc., West Chester, PA, BDH Inc., Toronto, Canada, Fluka Chemical Corp., Ronkonkoma, NY, Pfaltz & Bauer, Inc., Waterbury, CT, Avocado Research, Lancashire, UK, Crescent Chemical Co., Hauppauge,  
30 NY, Fisher Scientific Co., Pittsburgh, PA, Fisons Chemicals, Leicestershire, UK, ICN Biomedicals, Inc., Costa Mesa, CA, Pierce Chemical Co., Rockford, IL, Riedel de Haen

AG, Hannover, Germany, Wako Chemicals USA, Inc., Richmond, VA, Maybridge Chemical Co. Ltd., Cornwall, UK, Bionet, Cornwall, UK, Trans World Chemicals, Inc., Rockville, MD, Apin Chemicals Ltd., Milton Park, UK, and Parish Chemical Co., Orem, UT.

5                   Compounds of formula (1) can be synthesized using



10                   and  $\text{R}_1\text{-SO}_2\text{Cl}$  as starting materials. Examples of these syntheses are depicted in Figures 1A-B.

15                   The synthesis depicted in Figure 1A is performed as follows. The polystyrene resin, P, functionalized with a 3-benzyloxy-1,2-propanediol (5 mmol; 1 mmol/g; 5g) as described in Leznoff and Wong, *Can. J. Chem.*, **51**:3756-3764 (1973), is swollen in anhydrous acetone. Alternatively, functionalized resins can be purchased from Novabiochem. Then, 3-aminophenylboronic acid (10 mmol; 0.93g) is dissolved in anhydrous acetone and then added to the resin suspension, and the suspension is agitated. Anhydrous sodium sulfate can be used to absorb liberated water. Then, the resin is filtered and washed several times with water (to remove sodium sulfate and ethanol) and then with ether. The resin is divided into batches (20 mg of resin each). The resin is suspended in N-methyl pyrrolidone (NMP) and then the sulfonyl chloride (10 mmol), previously dissolved in NMP, is added to the suspension. Diisopropylamine (DIEA; 20 mmol) is added. The reactions are mixed for 3-4 hours. Then, excess reagents are washed away. The final products are cleaved from the resin using acidic conditions and the resin is then separated by filtration. The solution is evaporated or extracted to give the final product.

25                   The synthesis depicted in Figure 1B is performed as follows. First, 3-aminophenylboronic acid hemisulfate (20 mg; 0.11 mmol) is dissolved in  $\text{CHCl}_3$  (6 ml). (Piperidinomethyl)-polystyrene (0.33 mmol; 2.6 mmol/g; 0.127g) is added to the solution.  $\text{R}_1\text{-SO}_2\text{Cl}$  (0.165 mmol) is added, and the reaction agitated using a platform shaker until

the presence of starting material is no longer detected by thin layer chromatography (TLC) (3-4 hours). Then, aminomethylated-polystyrene (0.28 mmol; 1 mmol/g; 0.28g) is added to scavenger the excess thionyl chloride. The reaction is agitated for another 3-4 hours. The reaction is then filtered and concentrated to give the final product.

5           The compounds of formula (1) may contain an acidic or basic functional group and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids and bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic acid and base addition salts of compounds of formula (1). These salts can be prepared by reacting the purified  
10       compound with a suitable acid or base. Suitable bases include the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, ammonia, or a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation  
15       of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. Representative acid addition salts include the hydrobromide, hydrochloride, sulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthalate, mesylate, glucoheptonate, lactobionate, and  
20       laurylsulphonate salts and the like.

          The compounds of formula (1), and the pharmaceutically-acceptable salts thereof, are inhibitors of  $\beta$ -lactamases. The compounds of formula (1) may also prevent transcriptional up-regulation of  $\beta$ -lactamases and may be antibacterial by themselves, since it is likely that they will bind to PBPs which resemble  $\beta$ -lactamases.

25           Assays for the inhibition of  $\beta$ -lactamase activity are well known in the art. For instance, the ability of a compound to inhibit  $\beta$ -lactamase activity in a standard enzyme inhibition assay may be used (see, e.g., Example 2 below and M.G. Page, *Biochem J.* 295 (Pt. 1) 295-304 (1993)).  $\beta$ -lactamases for use in such assays may be purified from bacterial sources or, preferably, are produced by recombinant DNA techniques, since  
30       genes and cDNA clones coding for many  $\beta$ -lactamases are known. See, e.g., S.J. Cartwright and S.G. Waley, *Biochem J.* 221, 505-512 (1984). Alternatively, the

sensitivity of bacteria known, or engineered, to produce a  $\beta$ -lactamase may be determined. Other bacterial inhibition assays include agar disk diffusion and agar dilution. See, e.g., W.H. Traub & B. Leonhard, *Chemotherapy* 43, 159-167 (1997). Inhibition includes both reduction and elimination of  $\beta$ -lactamase activity.

5           The compounds of formula (1) may also be effective against bacteria resistant to  $\beta$ -lactam antibiotics as a result of porin mutations. Porin mutations are mutations in the genes that encode the proteins that form porin channels in bacterial cell walls. These mutations reduce the ability of  $\beta$ -lactam antibiotics to enter bacterial cells in which the mutations occur, thereby making the bacteria resistant to these antibiotics. The presence  
10 of a porin mutation can be detected by polymerase chain reaction analysis of porin genes, polyacrylamide gel electrophoresis of a preparation obtained by mild osmotic shock (e.g., treatment with hypotonic solution containing EDTA, followed by gentle centrifugation and separation of the supernatant) of the bacteria (absence of a protein of the appropriate molecular weight being indicative of a porin mutation), or by determining resistance to  
15 infection by bacteriophage Tu1A (a standard test for OmpF<sup>-</sup> porin mutations).

          The compounds of formula (1), or pharmaceutically-acceptable salts thereof, can be used to treat  $\beta$ -lactam-antibiotic-resistant bacterial infections. " $\beta$ -lactam-antibiotic-resistant bacterial infection" is used herein to refer to an infection caused by bacteria resistant to treatment with  $\beta$ -lactam antibiotics due primarily to the action of a  $\beta$ -  
20 lactamase. Resistance to  $\beta$ -lactam antibiotics can be determined by standard antibiotic sensitivity testing. The presence of  $\beta$ -lactamase activity can be determined as is well known in the art (see above). Alternatively, and preferably, the sensitivity of a particular bacterium to the combination of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, and a  $\beta$ -lactam antibiotic can be determined by standard antibiotic  
25 sensitivity testing methods.

          To treat a  $\beta$ -lactam-antibiotic-resistant bacterial infection, an animal suffering from such an infection is given an effective amount of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, and an effective amount of a  $\beta$ -lactam antibiotic. The compound of formula (1), or a pharmaceutically-acceptable salt thereof, and the  
30 antibiotic may be given separately or together. When administered together, they may be

contained in separate pharmaceutical compositions or may be in the same pharmaceutical composition.

Many suitable  $\beta$ -lactam antibiotics are known. These include cephalosporins (*e.g.*, cephalothin), penicillins (*e.g.*, amoxicillin), monobactams (*e.g.*, aztreonam), carbapenems (*e.g.*, imipenem), carbacephem (loracarbef), and others.  $\beta$ -lactam antibiotics are effective (in the absence of resistance) against a wide range of bacterial infections. These include those caused by both gram-positive and gram-negative bacteria, for example, bacteria of the genus *Staphylococcus* (such as *Staphylococcus aureus* and *Staphylococcus epidermis*), *Streptococcus* (such as *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus faecalis*), *Micrococcus* (such as *Micrococcus luteus*), *Bacillus* (such as *Bacillus subtilis*), *Listeria* (such as *Listeria monocytogenes*), *Escherichia* (such as *Escherichia coli*), *Klebsiella* (such as *Klebsiella pneumoniae*), *Proteus* (such as *Proteus mirabilis* and *Proteus vulgaris*), *Salmonella* (such as *Salmonella typhosa*), *Shigella* (such as *Shigella sonnei*), *Enterobacter* (such as *Enterobacter aerogenes* and *Enterobacter facium*), *Serratia* (such as *Serratia marcescens*), *Pseudomonas* (such as *Pseudomonas aeruginosa*), *Acinetobacter* such as *Acinetobacter anitratus*, *Nocardia* (such as *Nocardia autotrophica*), and *Mycobacterium* (such as *Mycobacterium fortuitum*). Effective doses and modes of administration of  $\beta$ -lactam antibiotics are known in the art or may be determined empirically as described below for the compounds of formula (1).

To treat an animal suffering from a  $\beta$ -lactam-antibiotic-resistant bacterial infection, an effective amount of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, is administered to the animal, in combination with a  $\beta$ -lactam antibiotic. Effective dosage forms, modes of administration and dosage amounts of a compound of formula (1), may be determined empirically, and making such determinations is within the skill of the art. It is understood by those skilled in the art that the dosage amount will vary with the activity of the particular compound employed, the severity of the bacterial infection, the route of administration, the rate of excretion of the compound, the duration of the treatment, the identity of any other drugs being administered to the animal, the age, size and species of the animal, and like factors well known in the medical and veterinary arts. In general, a suitable daily dose will be that amount which is the lowest dose effective to produce a therapeutic effect. The total daily dosage will be determined by an attending

physician or veterinarian within the scope of sound medical judgment. If desired, the effective daily dose of a compound of formula (1), or a pharmaceutically-acceptable salt thereof, may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day. Treatment of a  $\beta$ -lactam-antibiotic-resistant bacterial infection according to the invention, includes mitigation, as well as elimination, of the infection.

Animals treatable according to the invention include mammals. Mammals treatable according to the invention include dogs, cats, other domestic animals, and humans.

Compounds of formula (1) or pharmaceutically-acceptable salts thereof, may be administered to an animal patient for therapy by any suitable route of administration, including orally, nasally, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually. The preferred routes of administration are orally and parenterally.

While it is possible for the active ingredient(s) (one or more compounds of formula (1), or pharmaceutically-acceptable salts thereof, alone or in combination with a  $\beta$ -lactam antibiotic) to be administered alone, it is preferable to administer the active ingredient(s) as a pharmaceutical formulation (composition). The pharmaceutical compositions of the invention comprise the active ingredient(s) in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient.

Pharmaceutical formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. Regardless of the route of administration selected, the active ingredient(s) are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

The amount of the active ingredient(s) which will be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration and all of the other factors described above. The amount of the active ingredient(s) which will be combined with a carrier material to

produce a single dosage form will generally be that amount of the active ingredient(s) which is the lowest dose effective to produce a therapeutic effect.

Methods of preparing pharmaceutical formulations or compositions include the step of bringing the active ingredient(s) into association with the carrier and, optionally,  
5 one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active ingredient(s) into association with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and  
10 acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of the active ingredient(s). The active ingredient(s) may also be administered as a bolus,  
15 electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient(s) is/are mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose,  
20 sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as  
25 quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering  
30 agents. Solid compositions of a similar type may also be employed as fillers in soft and

hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

5 A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient(s) moistened with an inert liquid diluent.

10 The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient(s) therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release  
15 profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used  
20 include polymeric substances and waxes. The active ingredient(s) can also be in microencapsulated form.

Liquid dosage forms for oral administration of the active ingredient(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups  
25 and elixirs. In addition to the active ingredient(s), the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame  
30 oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

5        Suspensions, in addition to the active ingredient(s), may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

10       Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing the active ingredient(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or salicylate and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active ingredient(s). Formulations of the present invention which are suitable for vaginal administration also  
15       include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

20       Dosage forms for the topical or transdermal administration of the active ingredient(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active ingredient(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required.

25       The ointments, pastes, creams and gels may contain, in addition to the active ingredient(s), excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

30       Powders and sprays can contain, in addition to the active ingredient(s), excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of the active ingredient(s) to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating the active ingredient(s) in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the active ingredient(s) across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the active ingredient(s) in a polymer matrix or gel.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise the active ingredient(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the active ingredient(s), it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient(s) then depends upon its/their rate of dissolution which, in turn, may depend upon crystal

size and crystalline form. Alternatively, delayed absorption of parenterally-administered active ingredient(s) is accomplished by dissolving or suspending the active ingredient(s) in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the active ingredient(s) in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of the active ingredient(s) to polymer, and the nature of the particular polymer employed, the rate of release of the active ingredient(s) can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the active ingredient(s) in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

The pharmaceutical compositions of the present invention may also be used in the form of veterinary formulations, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules or pellets for admixture with feed stuffs, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension or, when appropriate, by intramammary injection where a suspension or solution is introduced into the udder of the animal via its teat; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally, for example, as a pessary, cream or foam.

EXAMPLESEXAMPLE 1: Synthesis of Compounds

Compounds 1-14 listed in Table 1B were synthesized as follows. See also the description above and Figure 1B.

5           The syntheses were carried out in liquid phase in parallel on a ten-position shaker. For the synthesis of a single compound, 0.030 g (0.161 mmol) of 3-aminophenylboronic acid hemisulfate (Aldrich) was dissolved in anhydrous chloroform ( $\text{CHCl}_3$ ; 6 mL), and the solution was stirred at room temperature. Then, 3 eq. (0.483 mmol; 2.6 mmol/g; 0.186 g)(piperidinomethyl)-polystyrene resin (cross-linked with 2% divinylbenzene; 220-400  
10 mesh; purchased from Fluka) was added as base to scavenge the HCl formed during the reaction. Different sulfonyl chlorides ( $\text{R}_1\text{SO}_2\text{Cl}$ ; 1.5 eq., 0.241 mmol) (purchased from Aldrich, Maybridge International, TCI-US, Lancaster and Fluka) were then added, and the mixtures stirred until thin-layer chromatography (TLC) no longer detected the presence of starting material (from 12 to 24 hours). Then aminomethylated polystyrene  
15 resin (3 eq., 0.483 mmol; 1.33 mmol/g; 0.363 g; purchased from Novabiochem) was added to the reaction solutions to scavenge the excess sulfonyl chloride, and stirring was continued for 4-5 hours. Finally, the resins were filtered off, and the filtrate was concentrated to give the final compound.

20           Compounds 1 and 3 in Table 1B were purified by column chromatography (Silica gel 60M; 230-400 mesh; eluent system -  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 9:1).

          Compounds 2, 4, 5, 6, 8, and 10 were separated from starting materials as follows. The solution was acidified with hydrochloric acid (4N HCl) and then extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) three times to remove the unreacted 3-aminophenyl boronic acid. The combined organic layers were dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and the  
25 solvent was removed under vacuum. The collected product was then triturated and washed with ethyl ether.

          Compounds 7, 12 and 14 were extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) at basic pH (4N NaOH) to hydrolyze and then remove the starting sulfonyl chloride present in the mixture. The combined organic layers were concentrated under vacuum. Upon  
30 acidification with 4N hydrochloric acid, a second extraction with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) was performed to remove the unreacted 3-aminophenyl boronic acid. The

combined organic layers were dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and the solvent was removed under vacuum. The collected product was then triturated with ethyl ether.

The purity of all synthesized compounds was determined by TLC using silica gel 60 F<sub>254</sub> plates (Merck) with the appropriate solvent system. The chromatograms were visualized using a UV/visible lamp at  $\lambda$  254 nm and 366 nm. The structures of the most active compounds were characterized by NMR spectroscopy and/or mass spectrometry.

The purity and yields of the compounds listed in Table 1B are given in the following table.

Compound	Purity %	Chloride eq.*	Yield%
1	98	1.5	24
2	60	3	32
3	80	1.5	1
4	90	3	13
5	90	3	6
6	60	1.5	5
7	80	3	8
8	70	3	21
9	80	3	14
10	70	3	8
11	70	4.5	8
12	70	3	9
13	90	3	38
14	70	3	4

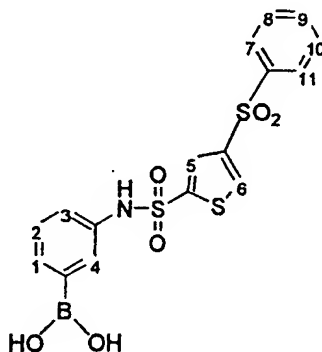
\*Chloride eq. = equivalents of sulfonyl chloride used for the synthesis.

Compound 1 has been also synthesized by traditional liquid phase method and in larger amount. First, 0.3 g (1.61 mmol) of 3-aminophenylboronic acid hemisulfate was dissolved in chloroform ( $\text{CHCl}_3$ , 30 mL) followed by pH adjustment to 10 using sodium hydroxide (4N NaOH). While stirring the solution at room temperature, 4-(benzene-sulfonyl)-thiophene-2-sulphonyl chloride (1.5 eq; 2.415 mmol, 0.779 g) dissolved in acetone (10 mL) was added. After stirring overnight, the reaction solution was acidified with hydrochloric acid (4N HCl) and extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) three times. The combined organic layers were dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and the

solvent was removed under vacuum. The collected crude product was then crystallized from acetone:petroleum ether, 60:80. The yield was 24%.

The <sup>1</sup>H-NMR data for compound 1 were:

5



p.n.	δ(ppm)
H1	7.650 d
H2	7.293 t
H3	7.175 dd
10 H4	7.613 s
H5	7.783 s
H6	8.750 s
H7-H11	9.000 d
H8-H10	7.725 t
15 H9	7.830 t
SO <sub>2</sub> NH	10.537 s
OH	8.173 s (2 protons)
Correlation Cosy: H1-H2; H2-H3; H7-H8; H8-H9	

20

#### EXAMPLE 2: Synthesis Of Compounds

Several additional compounds were synthesized. Their structures are given in Table 1B below (compounds 15-26). The synthetic scheme for these compounds is illustrated in Figure 2 and described in this example.

25

3-(4-Benzenesulfonyl-thiophene-2-sulfonylamino)-phenyl boronic acid (**1**): 0.3 g (1.61 mmol) of 3-aminophenylboronic acid hemisulfate (3APB) was dissolved in chloroform (CHCl<sub>3</sub>, 30 mL) followed by pH adjustment to 10 using sodium hydroxide (4N NaOH). To the solution, kept under stirring at room temperature, was then added 4-(benzene-sulfonyl)-thiophene-2-sulfonyl chloride (1.5 eq; 2.415 mmol, 0.779 g) already dissolved in acetone (10 mL). After stirring overnight, the reaction solution was acidified

30

with hydrochloric acid (4N HCl) and extracted with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) three times. The combined organic layers were dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and the solvent removed under vacuum. The collected crude product was then crystallized from acetone/petroleum ether 60:80. Yield 0.163 g, 24%.

5           3-(3,5-Dimethyl-isoxazole-4-sulfonylamino)-phenyl boronic acid (2): 0.2 g (1.075 mmol) of 3APB was dissolved in sodium bicarbonate (0.5 M,  $\text{NaHCO}_3$ , 20 mL), followed by pH adjustment to 10 using sodium hydroxide (4N NaOH). To the solution, kept under stirring at  $30^\circ\text{C}$ , was then added 3,5-dimethyl-isoxazole-4-sulphonyl chloride (1.5 eq; 1.61 mmol, 0.315 g) already dissolved in acetone (10 mL). After stirring 4-5 hours, the  
10 insoluble portion was filtered off, and the clear solution was acidified with hydrochloric acid (4N HCl) and extracted with ethyl-acetate, three times (15 mL each). The combined organic layers were dried over sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and the solvent was removed under vacuum. The collected product was then triturated with ethyl ether. Yield 0.124 g, 39%.

15           3-(5-Benzenesulfonyl-thiophene-2-sulfonylamino)-phenyl boronic acid (3): Compound 3 was prepared according to the method described for 2 starting from 0.2 g (1.075 mmol) of 3APB and 5-benzenesulfonyl-thiophene-2-sulfonyl chloride (1.5 eq; 1.61 mmol, 0.521 g). Yield 0.140 g, 31%.

20           3-(5-Chloro-4-nitro-thiophene-2-sulfonylamino)-phenyl boronic acid (23): Compound 23 was prepared according to the method described for 2 starting from 3APB (0.2 g, 1.075 mmol) and 5-chloro-4-nitro-thiophene-2-sulfonyl chloride (0.423 g, 1.5 eq.). The final crude product, 0.144 g, was purified by column chromatography, using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield 0.08 g, 21%.

25           3-{5-[(4-Chloro-benzoylamino)-methyl]-thiophene-2-sulfonylamino}-phenyl boronic acid (24): Compound 24 was prepared according to the method described for 2 starting from 3APB (0.1 g, 0.58 mmol) and 5-[(4-chloro-benzoylamino)-methyl]-thiophene-2-sulfonyl chloride (0.282 g, 1.5 eq). The final crude product, 0.240 g, was purified by column chromatography with  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield 0.07 g, 27%.

30           3-{5-[(benzoylamino)-methyl]-thiophene-2-sulfonylamino}-phenyl boronic acid (25): Compound 25 was prepared according to the method described for 2 starting from

3APB (0.1 g, 0.58 mmol) and 5-(benzoylamino)-methyl-thiophene-2-sulfonyl chloride (0.255 g, 1.5 eq). Yield 0.180 g, 80%.

3,3'-(1,3"-benzenedisulfonamide) phenyl boronic acid (15): Compound 15 was prepared according to the method described for 2 starting from 1,3-benzenedisulfonyl chloride (0.363 mmol, 0.1 g) and 3APB (0.141 g, 0.762 mmol). The final product was purified by column chromatography using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  95:5 as eluent. Yield: 0.083 g, 64%.

3-[3,5-Dichloro-4-(2-chloro-4-nitro-phenoxy)-benzenesulfonylamino]-phenyl boronic acid (17): Compound 17 was prepared according to the method described for 2 starting from 3APB (0.08 g, mmol) and 3,5-dichloro-4-(2-chloro-4-nitro-phenoxy)-benzenesulfonyl chloride (0.359 g, 2 eq.). The final crude product, 0.2 g, was purified by column chromatography using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield: 0.090 g, 40%.

3-[3-(4-carboxy-benzoylamino)-benzenesulfonylamino]-phenyl boronic acid (19): Compound 19 was prepared according to the method described for 2 starting from 3APB (0.15 g, 0.806 mmol) and 3-(4-carboxy-benzoylamino)-benzene sulfonyl chloride (1.21 g, 1.5 eq.). The crude product was purified by column chromatography using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield 0.06 g, 18%.

3-(4-carboxy-benzenesulfonylamino)-phenyl boronic acid (20): Compound 20 was prepared according to the method described for 2 starting from 3APB (0.1 g, 0.54 mmol) and 4-(chlorosulfonyl) benzoic acid (0.355 g, 3 eq). Yield: 0.046 g, 27%.

3-(3-Nitro-benzenesulfonylamino)-phenyl boronic acid (21): Compound 21 was prepared according to the method described for 2 starting from 3APB (0.2 g, 1.075 mmol) and 3-nitro-benzene sulfonyl chloride (0.357 g, 1.5 eq.). Yield: 0.180 g, 52%.

3-(4-Nitro-benzenesulfonylamino)-phenyl boronic acid (26): Compound 26 was prepared according to the method described for 2 starting with 3APB (0.4 g, 2.15 mmol) and 4-nitro-benzene sulfonyl chloride (0.477 g, 1 eq.). Yield: 0.559 g, 81%.

3-(3-Amino-benzenesulfonylamino)-phenyl boronic acid (22): The starting nitro derivative (21) (0.1 g, 0.311 mmol) was dissolved in a mixture of water (30 mL) and methanol (10mL). The catalyst, Pd/C (13%, 0.013 g), was added to the solution, transferred to a reactor and stirred under 2 atm of  $\text{H}_2$  for 1 hour. At the end of the reaction, the catalyst was filtered off and the solution concentrated under vacuum, giving

the pure reduced amino derivatives. Yield: 0.070 g, 77%. Compound (40) was also prepared in this same manner starting with compound (26).

3-[3-(4-carboxy-benzenesulfonylamino)-benzenesulfonylamino]-phenyl boronic acid (16): Compound 16 was prepared according to the method described for 2 starting from 3-(3-amino-benzenesulfonylamino)-boronic acid (0.04 g, 0.137 mmol) (22) and 4-carboxy-benzenesulfonyl chloride (0.045 g, 1.5 eq). The final product, 0.015 g, was purified through column chromatography using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield: 0.008 g, 12%.

3-[4-(4-carboxy-benzenesulfonylamino)-benzenesulfonylamino]-phenyl boronic acid (18): Compound 18 was prepared according to the method described for 2 starting from 3-(4-amino-benzenesulfonylamino)-phenyl boronic acid (0.1 g, 0.343 mmol) (40) and 4-carboxy-benzenesulfonyl chloride (0.113 g, 1.5 eq.). The final product, 0.143 g, was purified by column chromatography using  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  9:1 as eluent. Yield: 0.019 g, 12%.

### EXAMPLE 3: Testing Of Compounds For Inhibition Of $\beta$ -Lactamases

Compounds were tested for inhibition of TEM-1 and AmpC  $\beta$ -lactamases from *E. coli* using a spectrophotometric assay (Page, *Biochem. J.*, 295, 295-304 (1993)).

AmpC was expressed in *E. coli* JM109 cells (available from American Type Culture Collection, Rockville, MD, accession no. 53323) in which the native AmpC gene was attenuated or completely removed (obtained from Larry Blaszcak, Eli Lilly and Co., Indianapolis, Indiana) as described in Usher et al., *Biochemistry*, 37, 16082-16092 (1998). Briefly, DNA coding for the enzyme was located on a plasmid under the control of a temperature sensitive repressor. Cells containing this plasmid were grown in 2 liters of LB broth in a fermentor to log phase. Enzyme expression was then induced by temperature shock, and the cells were allowed to grow overnight. AmpC protein was purified from the supernatant over an Affigel-10 aminophenyl boronate affinity column (Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA). The purity of the sample was estimated by HPLC to be 96% or better. The amount of enzyme produced was estimated to be 150 mg based on absorbance at 280 nm.

TEM-1 was provided by Natalie Strynadka, University Of Alberta, Edmonton, Canada. Alternatively, TEM-1 may be produced as follows. The TEM-1 gene is cloned into HpaI site of pALTER-EX2 (Promega). The gene is under control of the T7 promoter which is turned on for protein expression. TEM-1 may be expressed in JM109 cells, as well as several other *E. coli* strains. Cells are grown to late log phase, followed by induction of protein expression. The cells are spun down and the supernatant, into which the enzyme has been exported, is collected. Because the enzyme has been exported into the supernatant, purification may be achieved using standard column chromatography, as described in Matagne *et al. Biochem J.* **265**, 131-146 (1990); Escobar *et al, Biochemistry* **33**, 7619-7626 (1994).

Initial stock solutions of 1-100 mM concentrations of each compound to be tested were prepared in DMSO (dimethyl sulfoxide). Solubility and absorbance profiles were determined by incremental addition of small volumes of DMSO stock solutions to assay buffer (50 mM phosphate, pH 7.0) at 25° C using an HP8543 UV/Visible spectrophotometer with multi-cell transport running HP ChemStation software (version 2.5). Enzymatic testing was typically started at an upper concentration limit determined by the solubility and absorbance profile of the compound.

Standard assay conditions for AmpC were as follows: pH 7.0; 100  $\mu$ M cephalothin, sodium salt, as substrate; reaction monitored at 265 nm (cephalothin  $\beta$ -lactam absorbance peak); T = 25° C; 50 mM phosphate buffer; no incubation of inhibitor with enzyme; cycle times of 10-15 seconds; total reaction volume = 1 mL; run time = 5 minutes; reaction initialized with addition of 0.06 nM AmpC. The background rate of cephalothin hydrolysis under these conditions was found to be two to three orders of magnitude less than the rate of the enzyme-mediated cephalothin hydrolysis, so no correction for background hydrolysis of substrate was used. For TEM-1, 100  $\mu$ M 6- $\beta$ -furylacryloylamidopenicillanic acid, triethylammonium salt (FAP), was used as the substrate, the reaction was monitored at 340 nm (FAP  $\beta$ -lactam absorbance peak) and the cycle time was increased to 25 seconds (since this substrate was somewhat light sensitive). Due to the light sensitivity of FAP, the background rate of hydrolysis for this substrate was found to be minimal, but not insignificant, so all measured control and inhibited cell rates were corrected by subtraction of the FAP background rate. All other conditions for

the TEM-1 assays were identical to those for the AmpC assays. DMSO was added to enzyme controls in all cases. Standard 1 mm path length quartz spectrophotometric cells (Hellma Cells, Inc., Jamaica, NY) were used in the assays. All assays were performed on the same HP8543 spectrophotometer noted earlier.

5           Linear and quadratic fits to the absorbance data for the full time course of each reaction were used to determine the reaction rate for each spectrophotometric cell. The resulting reaction rate data were used to calculate the inhibition constants for each potential inhibitor using the method of Waley (S.G. Waley, *Biochem. J.* 205, 631-633 (1982)). Briefly, this method involves the use of the integrated Michaelis-Menten  
10           equation to calculate  $K_i$  values for enzyme inhibitors from a comparison of the reaction rates of uninhibited and inhibited enzymatic reactions.

          Specificity testing was performed by assaying the activity of an inhibitor against  $\alpha$ -chymotrypsin (bovine pancreatic),  $\beta$ -trypsin (bovine pancreatic), and elastase (porcine pancreatic). Substrates for  $\alpha$ -chymotrypsin (N-benzoyl-L-tyrosine ethyl ester, BTEE) and  
15            $\beta$ -trypsin (N-benzoyl-L-arginine ethyl ester, BAEE) were purchased from Sigma Chemical, St. Louis, MO. The elastase substrate used (elastase substrate 1, N $\alpha$ -methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide, was purchased from Calbiochem, San Diego, CA. All enzymes used for specificity testing were purchased from Sigma Chemical, St. Louis, MO. For  $\alpha$ -chymotrypsin, 3  $\mu$ l of a 1 mg/ml enzyme stock solution  
20           (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized by addition of 630  $\mu$ M BTEE from a DMSO stock solution. The reaction was performed at 25°C and monitored at 260 nm. For  $\beta$ -trypsin, 40  $\mu$ l of a 0.8 mg/ml enzyme stock solution (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized  
25           by addition of 600  $\mu$ M BAEE from a DMSO stock solution. For elastase, 50  $\mu$ l of a 1 mg/ml enzyme stock solution (50 mM phosphate buffer, pH 7) was incubated with the boronate being tested for 5 minutes; then the reaction was initialized by addition of 64  $\mu$ M elastase substrate 1 from a DMSO stock solution.

          The results of the testing are presented in Tables 1A and 1B below. Certain prior  
30           art compounds (Table 1A) were tested for comparative purposes. All of these compounds were obtained from Lancaster Synthesis, Windham, NH, except for MAPB, which was

obtained from Sigma, St. Louis, MO. All of these compounds were used as is with no additional purification or verification performed. The compounds listed in Table 1B were synthesized as described in Examples 1 and 2.

5

TABLE 1A

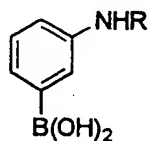
boronate	Ki <i>E. coli</i> AmpC ( $\mu$ M)	Ki <i>E. coli</i> TEM-1 ( $\mu$ M)
NSULFB	1.6	88.0
4FORMB	2.8	35.0
4MEPB	5.2	>100
MAPB	5.8	>>100
2FDB	8.0	>100
2FORMB	62.0	>100

15

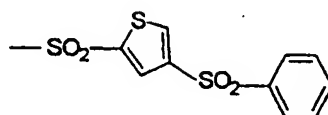
NSULFB = *m*-(dansylamidophenyl)-boronic acid; 4FORMB = 4-formylphenylboronate; 4MEPB = 4-methylphenylboronate; MAPB = *m*-aminophenylboronate; 2FDB = phenylboronic acid; and 2FORMB = 2-formylphenylboronate.

20

TABLE 1B

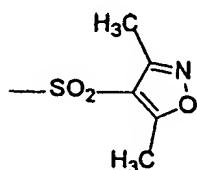
NumberRKi AmpC ( $\mu$ M)

1



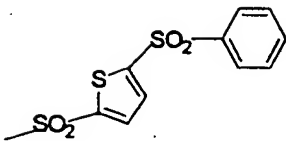
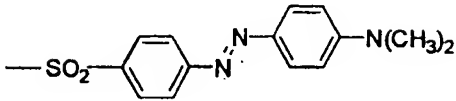
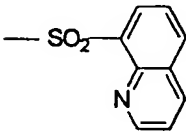
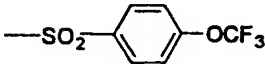
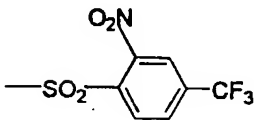
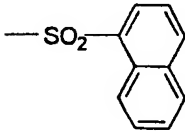
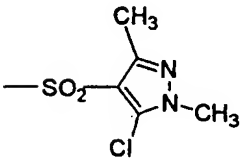
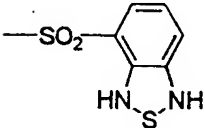
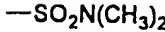
0.040

2

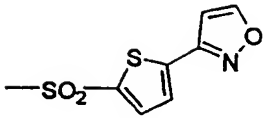
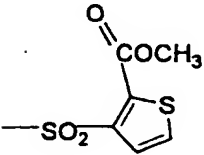
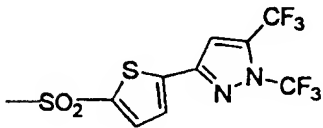
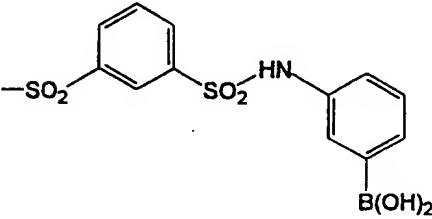
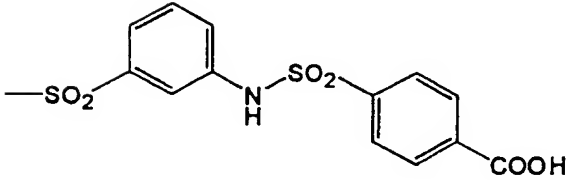
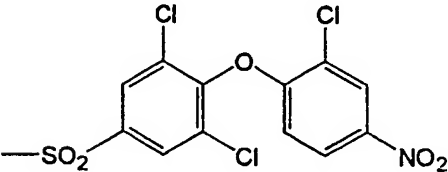


0.10

26

<u>Number</u>	<u>R</u>	<u>Ki AmpC (μM)</u>
3		0.12
4		0.53
5		0.66
6		0.70
7		0.82
8		1.3
9		1.6
10		1.6
11		5.5

27

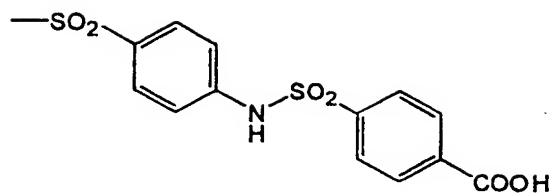
<u>Number</u>	<u>R</u>	<u>Ki AmpC</u> <u>(<math>\mu</math>M)</u>
12		12.3
13		13.2
14		15.5
15		0.06
16		0.08
17		0.20

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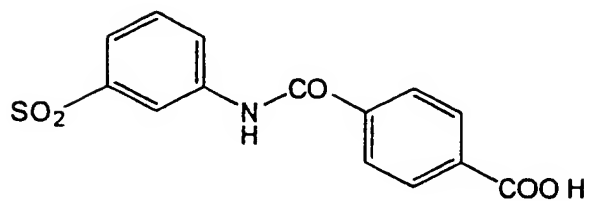
NumberRKi AmpC( $\mu$ M)

18



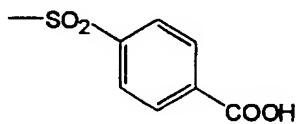
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19



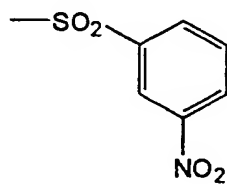
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20



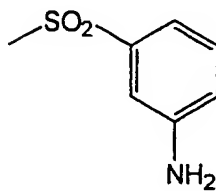
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21



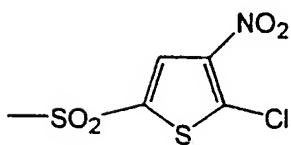
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22



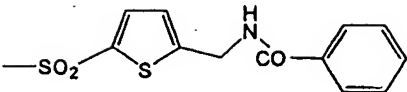
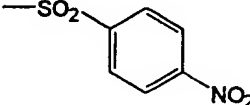
1.2

23



0.68

29

<u>Number</u>	<u>R</u>	<u>Ki AmpC (μM)</u>
25		1.02
26		0.77

**EXAMPLE 4: Testing of Compounds for Inhibition of Bacterial Growth**

Bacterial cell culture testing was performed and interpreted following the guidelines of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa. 1993). The tested bacterial strains were all clinical isolates from the Hospital Ramon y Cajal in Madrid, and all of the strains listed in Table 2 below are available from Jesus Blazquez and Fernando Baquero, Servicio de Microbiologia, Hospital Ramon y Cajal, National Institute of Health, Madrid, Spain. After incubation, the growth of the cells was visually inspected. The minimum inhibitory concentration (MIC) is the lowest concentration (μg/ml) where no cell growth was observed. The results are presented in Table 2 below.

TABLE 2

Minimum Inhibitory Concentrations (μg/ml)

Bacteria	Amx <sup>a</sup>	1 (alone)	16 (alone)	Clav <sup>a</sup> alone	Amx-1 <sup>b</sup>	Amx-16 <sup>b</sup>	Amx- Clav <sup>c</sup>
<i>S. epidermidis</i> 30995	64	192	48	32	8	4	2
<i>S. epidermidis</i> 31091	32	192	48	64	4	4	2
<i>S. aureus</i> 12	64	384	384	4	8	8	0.5
<i>S. aureus</i> 39	64	384	192	8	4	4	0.5
<i>S. aureus</i> 42	256	192	384	>256	16	16	16
<i>S. aureus</i> 44	128	384	192	16	4	4	1
<i>S. epidermidis</i> 1108	128	192	384	8	4	8	0.5

Bacteria	Amx <sup>a</sup>	1 (alone)	16 (alone)	Clav <sup>a</sup> alone	Amx-1 <sup>b</sup>	Amx-16 <sup>b</sup>	Amx- Clav <sup>c</sup>
<i>Micrococcus sp.</i> 3110925	256	96	96	>256	16	8	32
<i>Bacillus sp.</i> 70002	8	192	196	256	2	4	4
<i>Nocardia sp.</i>	>512	786	384	>256	128	256	64

a. Amx: amoxicillin; Clav: Clavulanic acid

b. Amoxicillin/Inhibitor = 1/3 (w/w)

c. Amoxicillin/Clav = 2/1 (w/w)

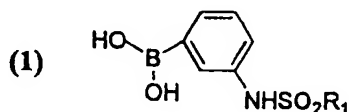
As noted, compounds 1 and 16 were tested at 3-fold higher concentrations than the MIC values for amoxicillin reported. Thus, a reported MIC of 4  $\mu\text{g/ml}$  for amoxicillin in Table 2 indicates that compound 1 or 16 was present at 12  $\mu\text{g/ml}$  (12  $\mu\text{g/ml}$  is approximately 30  $\mu\text{M}$ ; 24  $\mu\text{g/ml}$  is approximately 60  $\mu\text{M}$ , and so forth). These MIC values are consistent with the  $K_i$  values that were observed when the compounds were tested against the enzymes in isolation (MIC values should be higher the  $K_i$  values, due to the need to saturate the enzyme and cell-based barriers to entry).

The tested bacterial strains do not express class C (AmpC-like)  $\beta$ -lactamases (see Example 3). Instead, they express class A  $\beta$ -lactamases, most probably an enzyme that resembles the PCI  $\beta$ -lactamase from *Staphylococcus aureus*. Curiously, the tested bacteria are much more affected by these inhibitors than most bacteria expressing AmpC-like enzymes (data not shown). This probably reflects the fact that Gram positive bacteria lack an outer membrane, apparently a feature of Gram negative bacteria that seriously diminishes efficacy against them, and only Gram negative bacteria are known to express class C  $\beta$ -lactamases.

In view of the results in Table 2, compounds 1, 15, and 16, currently the best inhibitors, were tested against the PCI  $\beta$ -lactamase of *S. aureus* (a gift from Robert Bonomo, Case Western Reserve University, Cleveland, Ohio). This assay was performed as described in Example 3 for TEM-1. Compounds 1, 15 and 16 were found to inhibit the PCI  $\beta$ -lactamase at low  $\mu\text{M}$  concentrations (6  $\mu\text{M}$ , 3.2  $\mu\text{M}$ , and 2  $\mu\text{M}$ , respectively). This is consistent with their efficacy in synergizing  $\beta$ -lactam antibiotics (see Table 2).

## WE CLAIM:

1. A method of treating a  $\beta$ -lactam-antibiotic-resistant bacterial infection comprising administering to an animal suffering from such an infection an effective amount of a compound having the formula:



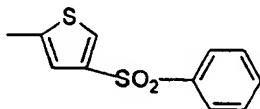
wherein:

$R_1$  is N-lower alkyl, a cyclic alkene or a heterocyclic alkene, wherein the cyclic alkene and heterocyclic alkene may be substituted with one or more substituents  $R_2$ ; and each  $R_2$  is independently  $H$ , a halogen atom, lower, alkyl, lower alkyl substituted with one or more halogen atoms,  $NH_2$ ,  $NO$ ,  $NO_2$ , N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms,  $OH$ , O-lower alkyl, O-lower alkyl substituted with one more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms,  $COOH$ , lower alkyl- $COOH$ ,  $COO$ -lower alkyl,  $CONH_2$ , CON-lower alkyl,  $SO_3H$ ,  $SO_2NH_2$ ,  $SO_2N$ -lower alkyl, or  $B(OH)_2$ , except that  $R_2$  cannot be N-lower alkyl when  $R_1$  is naphthalene;

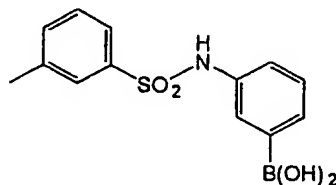
or a pharmaceutically-acceptable salt thereof; and

an effective amount of a  $\beta$ -lactam antibiotic.

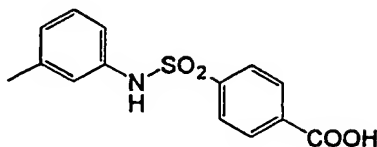
2. The method of Claim 1 wherein  $R_1$  is



3. The method of claim 1 wherein  $R_1$  is

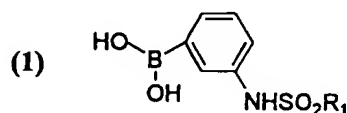


4. The method of Claim 1 wherein  $R_1$  is



5. The method of Claim 1 wherein the  $\beta$ -lactam antibiotic is amoxicillin or ceftazidime.

6. A method of inhibiting a  $\beta$ -lactamase comprising contacting the  $\beta$ -lactamase with an effective amount of a compound having the formula:



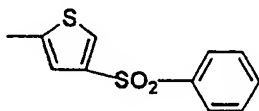
10 wherein:

$R_1$  is N-lower alkyl, a cyclic alkene or a heterocyclic alkene, wherein the cyclic alkene and heterocyclic alkene may be substituted with one or more substituents  $R_2$ ; and

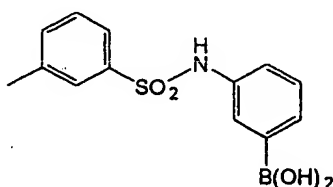
each  $R_2$  is independently H, a halogen atom, lower, alkyl, lower alkyl substituted with one or more halogen atoms,  $\text{NH}_2$ , NO,  $\text{NO}_2$ , N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms,  $\text{COOH}$ , lower alkyl- $\text{COOH}$ ,  $\text{COO}$ -lower alkyl,  $\text{CONH}_2$ ,  $\text{CON}$ -lower alkyl,  $\text{SO}_3\text{H}$ ,  $\text{SO}_2\text{NH}_2$ ,  $\text{SO}_2\text{N}$ -lower alkyl, or  $\text{B(OH)}_2$ , except that  $R_2$  cannot be N-lower alkyl when  $R_1$  is naphthalene;

20 or pharmaceutically acceptable salts thereof.

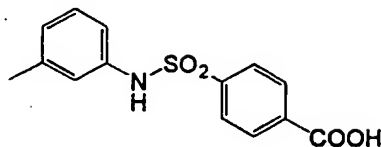
7. The method of Claim 6 wherein  $R_1$  is



- 25 8. The method of claim 6 wherein  $R_1$  is



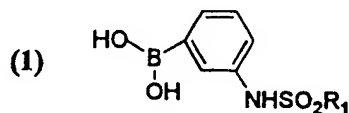
9. The method of Claim 6 wherein  $R_1$  is



10. The method of Claim 6 wherein the  $\beta$ -lactamase is produced by bacteria, and the bacteria are contacted with the compound or salt thereof.

11. The method of Claim 6 wherein the contacting takes place *in vitro*.

12. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier and a compound having the formula:

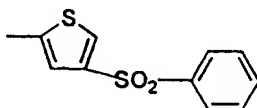


wherein:

- $R_1$  is N-lower alkyl, a cyclic alkene or a heterocyclic alkene, wherein the cyclic alkene and heterocyclic alkene may be substituted with one or more substituents  $R_2$ ; and each  $R_2$  is independently H, a halogen atom, lower, alkyl, lower alkyl substituted with one or more halogen atoms,  $NH_2$ , NO,  $NO_2$ , N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one or more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms, COOH, lower alkyl-COOH, COO-lower alkyl,  $CONH_2$ , CON-lower alkyl,  $SO_3H$ ,  $SO_2NH_2$ ,  $SO_2N$ -lower alkyl, or  $B(OH)_2$ , except that  $R_2$  cannot be N-lower alkyl when  $R_1$  is naphthalene; or

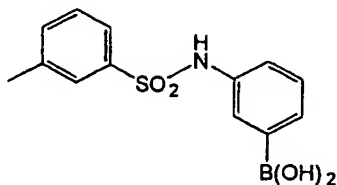
pharmaceutically-acceptable salts thereof.

13. The composition of Claim 12 wherein  $R_1$  is

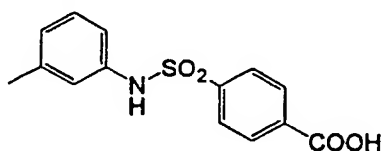


34

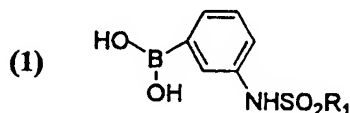
14. The composition of claim 12 wherein  $R_1$  is



- 5 15. The composition of Claim 12 wherein  $R_1$  is



16. The composition of Claim 12 further comprising a  $\beta$ -lactam antibiotic.  
 10 17. The composition of Claim 16 wherein the  $\beta$ -lactam antibiotic is amoxicillin or ceftazidime.  
 18. A compound having the formula:



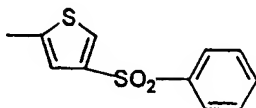
- 15 wherein:

$R_1$  is N-lower alkyl, a cyclic alkene or a heterocyclic alkene, wherein the cyclic alkene and heterocyclic alkene may be substituted with one or more substituents  $R_2$ ; and

- each  $R_2$  is independently H, a halogen atom, lower, alkyl, lower alkyl substituted with one or more halogen atoms,  $\text{NH}_2$ , NO,  $\text{NO}_2$ , N-lower alkyl, N-lower alkyl substituted with one or more halogen atoms, OH, O-lower alkyl, O-lower alkyl substituted with one more halogen atoms, CO-lower alkyl, CO-lower alkyl substituted with one or more halogen atoms,  $\text{COOH}$ , loweralkyl- $\text{COOH}$ ,  $\text{COO-lower alkyl}$ ,  $\text{CONH}_2$ , CON-lower alkyl,  $\text{SO}_3\text{H}$ ,  $\text{SO}_2\text{NH}_2$ ,  $\text{SO}_2\text{N-lower alkyl}$ , or  $\text{B(OH)}_2$ , except that  $R_2$  cannot be N-lower alkyl when  $R_1$  is naphthalene; or

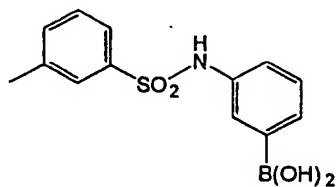
- 25 salts thereof.

19. The compound of Claim 18 wherein  $R_1$  is



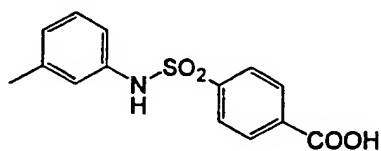
5

20. The compound of claim 18 wherein  $R_1$  is



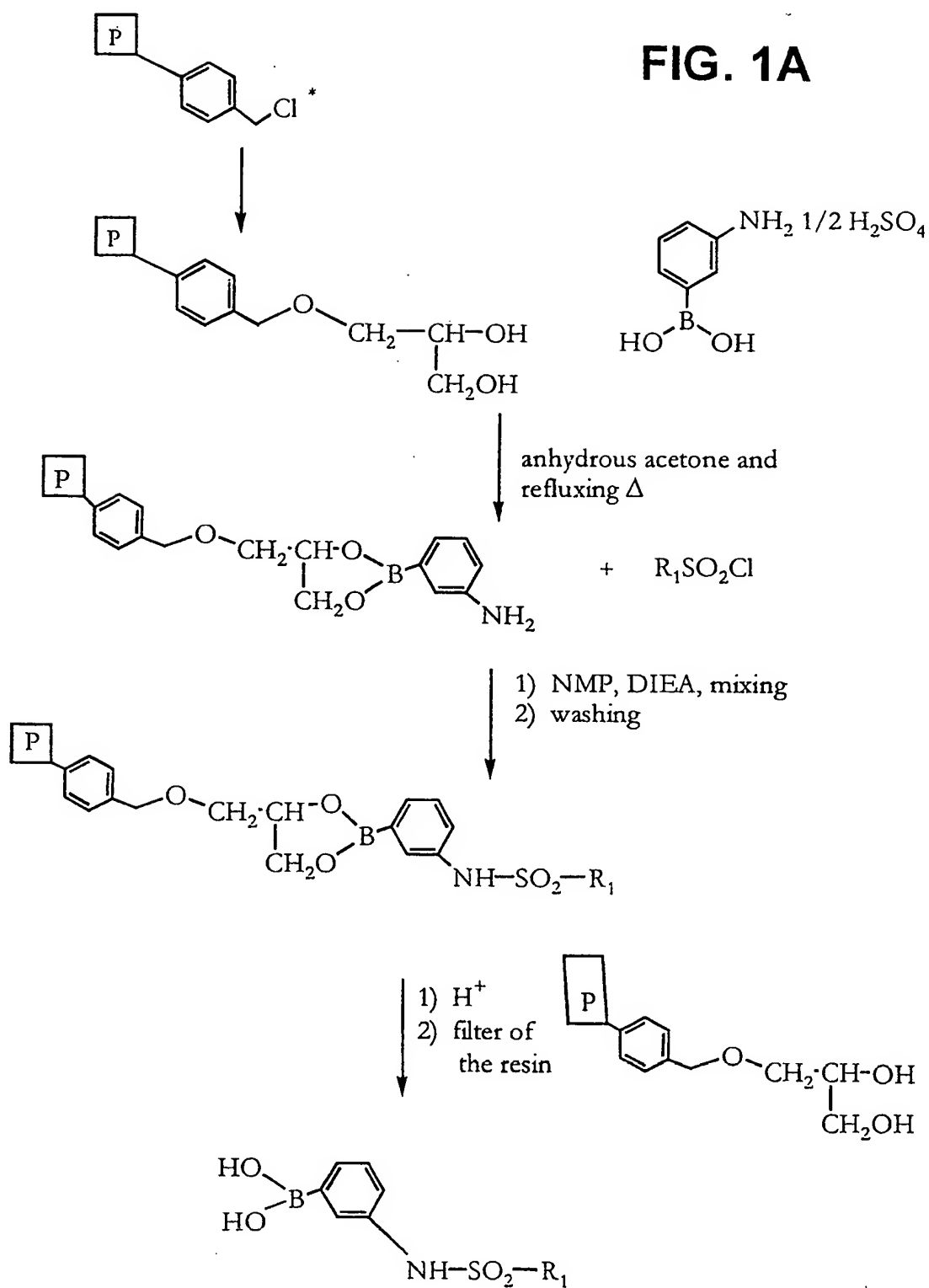
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21. The compound of Claim 18 wherein  $R_1$  is



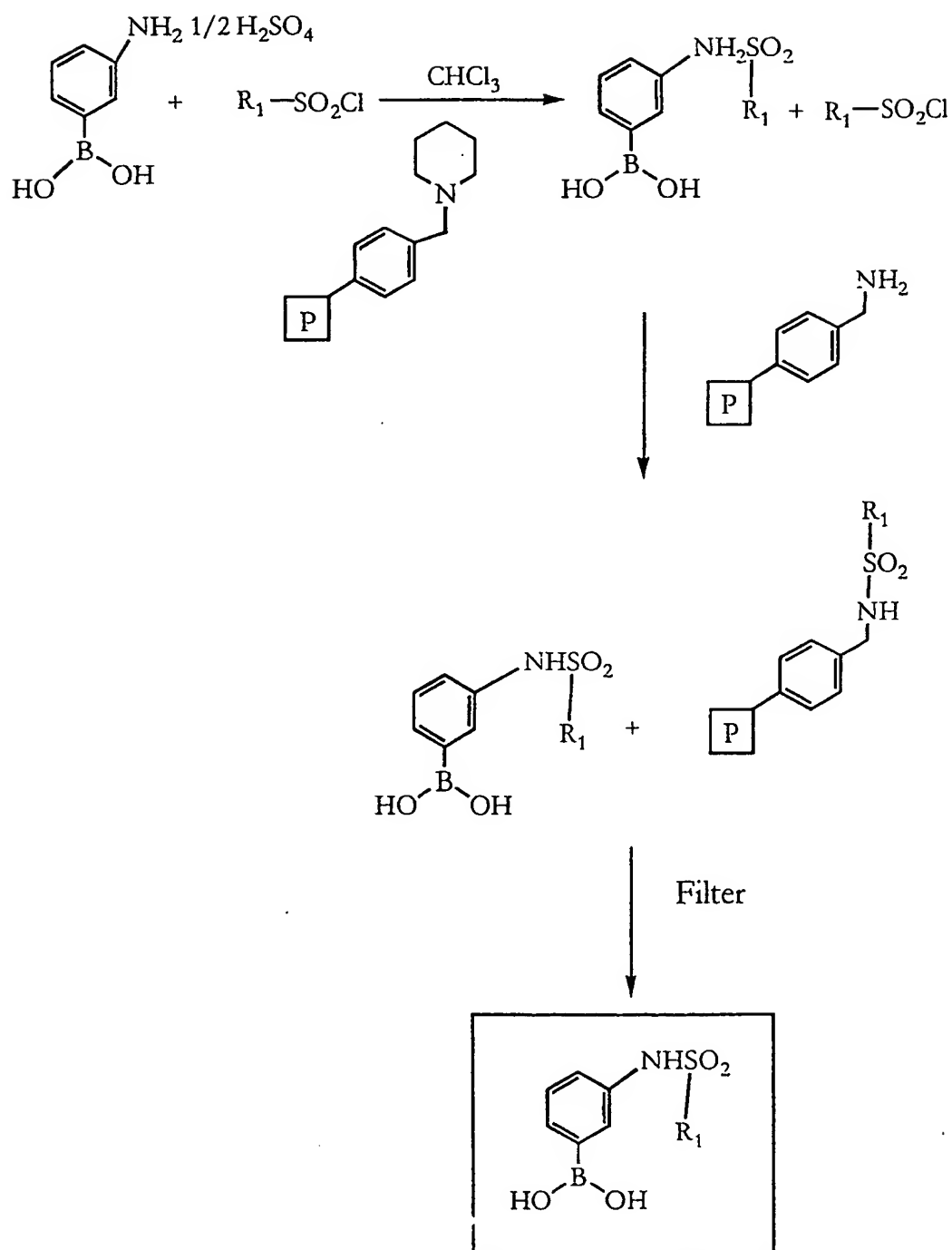
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FIG. 1A



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FIG. 1B



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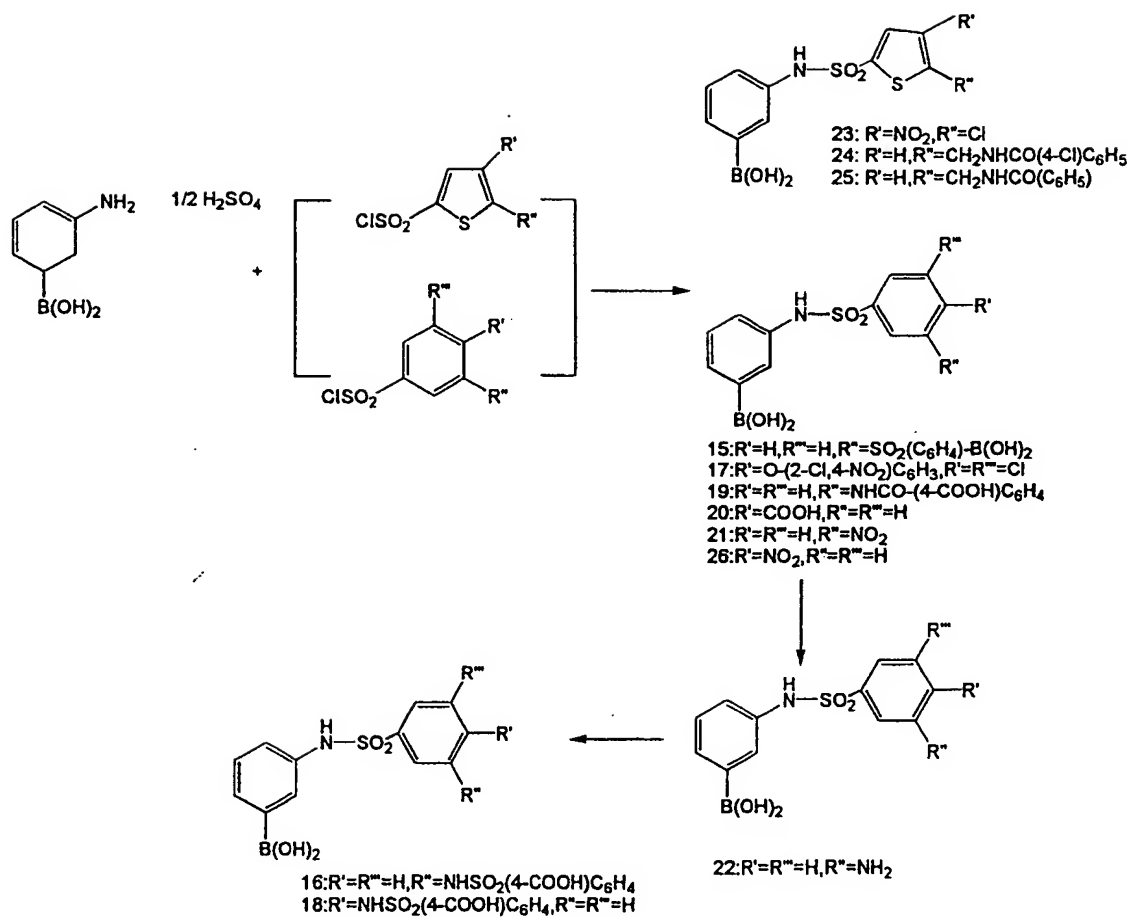


FIG. 2

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/29958

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) :C07D 333/32; C07C 303/00; A61K 31/38, 31/18 US CL :549/65; 564/82; 514/445, 603 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 549/65; 564/82; 514/445, 603 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,612,378 A (TIANBAO ET AL) 18 March 1997 (18/3/97), see abstract.	1-21
A,P	US 6,008,377 A (JONES ET AL) 28 DECEMBER 1999 (28/12/99), column 2, lines 35 to 45.	1-21
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier document published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel, or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *A* document member of the same patent family
Date of the actual completion of the international search 22 FEBRUARY 2000		Date of mailing of the international search report 21 MAR 2000
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